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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 030811woMetg	FOR FURTHER ACTION <small>See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA416)</small>	
International application No. PCT/EP 03/04058	International filing date (day/month/year) 17.04.2003	Priority date (day/month/year) 18.04.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant EVOTEC NEUROSCIENCES GMBH ET AL.		

<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 8 sheets.</p>
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the opinion II <input type="checkbox"/> Priority III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application

Date of submission of the demand 12.11.2003	Date of completion of this report 09.07.2004
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Aguilera, M Telephone No. +31 70 340-3897



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I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-34 as originally filed

Claims, Numbers

1-20 received on 06.05.2004 with letter of 06.05.2004

Drawings, Sheets

1/10-10/10 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:
- the drawings, sheets:

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5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).
(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:
 - the entire international application,
 - claims Nos. 9,10,12, 16-19
 - because:
 - the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):
 - the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 - the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 - no international search report has been established for the said claims Nos. 9,10,12, 16-19
2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
 - the written form has not been furnished or does not comply with the Standard.
 - the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	
	No: Claims	1-8, 11, 13-15 and 20
Inventive step (IS)	Yes: Claims	
	No: Claims	1-8, 11, 13-15 and 20
Industrial applicability (IA)	Yes: Claims	1-8, 11, 13-15 and 20
	No: Claims	

2. Citations and explanations

see separate sheet

V. Reasoned statement (Continuation)

2.1 CITATIONS

Reference is made to the following documents:

- D1: WO 00 55318 A (UNIV BRITISH COLUMBIA ;XENON BIORESEARCH INC (CA)) 21 September 2000
- D2: US-B1-6 225 525 (LEUNG WAI-PING ET AL) 1 May 2001
- D3: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 2001 (2001-07), VAISMAN BORIS L ET AL: 'ABCA1 overexpression leads to hyperalphalipoproteinemia and increased biliary cholesterol excretion in transgenic mice.' XP002237202 Database accession no. PREV200100378305 & JOURNAL OF CLINICAL INVESTIGATION, vol. 108, no. 2, July 2001 (2001-07), pages 303-309.
- D4: US 2002 037843 A1 (LAM FRED CHIU-LAI ET AL) 28 March 2002

2.2 NOVELTY (Art. 33(2) PCT)

- 2.2.1 D1 discloses methods and products for the diagnosis of neurodegenerative diseases, including Alzheimer's disease (see pages 46 to 51). It is explained that ABCA1 may act as a transporter of APP products out of cells, and therefore be useful in the context of amyloidosis. It is explicitly disclosed that down-regulation of ABCA1 may lead to decreased apoptosis in neurodegenerative disorders (see page 46, lines 22-25).
- 2.2.2 In particular, D1 describes a method of diagnosing a neurodegenerative disease by determining the level and/or activity of an ABCA1 gene product. Antibodies against ABCA1 can be used in the development of diagnostic assays by monitoring the expression or biological activity of ABCA1 (see page 48, lines 18-27). Other tools can be used to monitor said expression, for example RT-PCR (see page 49, line 9). It is also described a method of evaluating a treatment by measuring the levels of expression of an ABCA1 gene product (see page 49, lines 15-24). Therefore, the methods of claims 1-7 and 20 are not new.
- 2.2.3 The fact that D1 deals as well with other types of ABCA-1 related diseases is not relevant for the question of novelty, since the passages cited above are explicitly directed to neurodegenerative diseases, including Alzheimer's disease (see for

example page 46, line 6; or claim 34). Nevertheless, it must be noted that the reference to neurodegenerative diseases in the present claims is not restricted to Alzheimer's disease.

2.2.4 The kit of present claim 8 is not new because its single component, a reagent "that selectively detects a transcription product of the ABCA1 gene", was known at the time of filing. Any polynucleotide comprising any fragment of the ABCA1 gene falls under the scope of such definition, and examples can be found in D2 and D3, among others. The features characterizing the method in which the kit is to be used ("by detecting [...] health status") represent merely an indication of intended use in the context of a product claim. This IPEA considers the use of the product as an optional feature not limiting the scope of a product claim. Therefore, claim 8 is not new.

2.2.5 D2 discloses a recombinant non-human animal comprising a non-native gene sequence coding for ABCA1 which is *obtainable by* the process described in claim 11 (see Abstract and Figure 1). Therefore, claim 11 is not new. The fact that the recombinant animal is used as a model for other purposes is irrelevant to the question of novelty of a product claim (see above).

2.2.6 D1 discloses drug screens for modulators of neurodegenerative diseases, including Alzheimer's disease. The methods are based on the modulation of expression levels of ABCA1 gene products (see page 47, line 5, to page 48, line 17; claim 34), not only in cells (see page 54), but also in test animals (see page 59). Therefore, the methods of claims 13-15 are not new.

2.2.7 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1-8, 11, 13-15 and 20 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

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**2.3 SUFFICIENCY OF DISCLOSURE, CLARITY AND SUPPORT (Arts. 5 and 6
PCT)**

2.3.1 Article 6 PCT requires that the matter for which protection is sought be defined in the claims in a clear and concise manner, and that all the claims be supported by the description. The support by the description must be sufficiently clear for the skilled person to put the invention as claimed into practice without undue burden, i.e. no more than a reasonable amount of experimentation, and without applying inventive skill.

2.3.2 The current application documents demonstrate that ABCA1 mRNA is underexpressed in the post-mortem frontal cortex of Alzheimer patients, when compared to its expression in the post-mortem temporal cortex of the same patients. However, the scope of the subject-matter claimed covers the prognosis, diagnosis, monitoring and evaluation of any neurodegenerative disease by measuring the levels or activities of any genetic product of the ABCA1 gene. The discrepancy between the claimed scope of protection and the actual teaching of the description is evidenced by the following:

2.3.2.1 The fact that any translation product of the ABCA1 gene provides a reliable and reproducible marker for Alzheimer disease is not credible because the regulation of transcription does not necessarily produce an analogous regulation of the protein levels or activities; protein activity can be regulated at multiple further levels: splicing, translation, intracellular localization, modulation by ligands, degradation/turnover, etc. the application does not provide any scientific evidence that the protein levels of ABCA1 are also reduced in the frontal cortex of patients, let alone the expression of "fragments" and "variants", or their activities.

2.3.2.2 The very different nature and etiology of the wide range of diseases under the label of "neurodegenerative" makes extremely unlikely, in the absence of experimental support, that a down-regulation of the ABCA1 mRNA can be considered a reliable and reproducible marker for all of them.

2.3.2.3 The fact that ABCA1 mRNA is down-regulated in post-mortem tissue of Alzheimer patients when comparing frontal vs. temporal cortex does not necessarily mean that said levels will be regulated in the same manner in the

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body fluids of an Alzheimer patient when compared to a healthy person. First, the application does not provide any evidence that ABCA1 mRNA can be detected in body fluids or tissue samples which are suitable for prognosis, diagnosis, monitoring, etc. And second, if said mRNA could be detected, there is no evidence that it would be down-regulated when compared to the levels of a healthy person.

- 2.3.2.4 The fact that post-mortem tissue samples of Alzheimer patients show this differential mRNA expression does not imply that a person at risk of developing Alzheimer, but without any symptoms, will show the same effect, even if molecular analysis of his brain tissue was possible. There is no scientific evidence in the application in support of the prognostic methods.
- 2.3.3 Considering these facts, this IPEA has reasonable doubts concerning first, the reproducibility of the invention as claimed, and second, the burden of experimentation and inventive skill necessary to carry out the invention over the whole claimed area. Therefore, the present application cannot be considered sufficiently disclosed in the sense of Article 5 PCT. For the same reasons, and despite being formally supported by the description, the present claims cannot be considered supported in the sense of Article 6 EPC.

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CLAIMS

1. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:

determining a level and/or an activity of

- (i) a transcription product of the ABCA1 gene, and/or
- (ii) a translation product of the ABCA1 gene, and/or
- (iii) a fragment, or derivative, or variant of said transcription or translation product,

in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. A method of monitoring the progression of a neurodegenerative disease in a subject, comprising:

determining a level and/or an activity of

- (i) a transcription product of the ABCA1 gene, and/or
- (ii) a translation product of the ABCA1 gene, and/or
- (iii) a fragment, or derivative, or variant of said transcription or translation product,

in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby monitoring the progression of said neurodegenerative disease in said subject.

3. A method of evaluating a treatment for a neurodegenerative disease, comprising:

determining a level and/or an activity of

- (i) a transcription product of the ABCA1 gene, and/or
- (ii) a translation product of the ABCA1 gene, and/or

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(iii) a fragment, or derivative, or variant of said transcription or translation product.

in a sample from a subject being treated for said disease and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby evaluating said treatment for said neurodegenerative disease.

4. The method according to any of claims 1 to 3 wherein said neurodegenerative disease is Alzheimer's disease.

5. The method according to any of claims 1 to 4 wherein said sample comprises a cell, or a tissue, or a body fluid, in particular cerebrospinal fluid or blood.

6. The method according to any of claims 1 to 5 wherein said reference value is that of a level and/or an activity of

(i) a transcription product of the ABCA1 gene, and/or
(ii) a translation product of the ABCA1 gene, and/or
(iii) a fragment, or derivative, or variant of said transcription or translation product,

in a sample from a subject not suffering from said neurodegenerative disease.

7. The method according to any of claims 1 to 6 wherein an alteration in the level and/or activity of a transcription product of the gene coding for ABCA1 and/or a translation product of the gene coding for ABCA1 and/or a fragment, or derivative, or variant thereof, in a sample cell, or tissue, or body fluid, in particular cerebrospinal fluid, from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

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8. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease by:

detecting in a sample from said subject a varied level, or activity, or both said level and said activity of a transcription product and/or of a translation product of the ABCA1 gene - compared to a reference value representing a known health status; and said kit comprising:

at least one reagent which is selected from the group consisting of

- (i) reagents that selectively detect a transcription product of the ABCA1 gene and
- (ii) reagents that selectively detect a translation product of the ABCA1 gene.

9. A method of treating or preventing a neurodegenerative disease, in particular Alzheimer's disease, in a subject comprising administering to said subject in a therapeutically or prophylactically effective amount an agent or agents which directly or indirectly affect an activity and/or a level of

- (i) the ABCA1 gene, and/or
- (ii) a transcription product of the ABCA1 gene, and/or
- (iii) a translation product of the ABCA1 gene, and/or
- (iv) a fragment, or derivative, or variant of (i) to (iii).

10. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

- (i) the ABCA1 gene, and/or
- (ii) a transcription product of the ABCA1 gene, and/or
- (iii) a translation product of the ABCA1 gene, and/or
- (iv) a fragment, or derivative, or variant of (i) to (iii).

11. A recombinant, non-human animal comprising a non-native gene sequence coding for ABCA1 or a fragment, or a derivative, or a variant thereof, said animal being obtainable by:

- (i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and
- (ii) introducing said targeting construct into a stem cell of a non-human animal, and
- (iii) introducing said non-human animal stem cell into a non-human embryo, and
- (iv) transplanting said embryo into a pseudopregnant non-human animal, and
- (v) allowing said embryo to develop to term, and
- (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and
- (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

12. Use of the recombinant, non-human animal according to claim 11 for screening, testing, and validating compounds, agents, and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease.

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13. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

- (i) the ABCA1 gene, and/or
- (ii) a transcription product of the ABCA1 gene, and/or
- (iii) a translation product of the ABCA1 gene, and/or
- (iv) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

- (a) contacting a cell with a test compound;
- (b) measuring the activity and/or level of one or more substances recited in (i) to (iv);
- (c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and
- (d) comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

14. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

- (i) the ABCA1 gene, and/or
- (ii) a transcription product of the ABCA1 gene, and/or
- (iii) a translation product of the ABCA1 gene, and/or
- (iv) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

- (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);
- (b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

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- (c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched control animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;
- (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases or disorders.

15. The method according to claim 14 wherein said test animal and/or said control animal is a recombinant animal which expresses a gene coding for ABCA1, or a fragment, or a derivative, or a variant thereof, under the control of a transcriptional control element which is not the native ABCA1 gene transcriptional control element.

16. An assay for testing a compound, preferably for screening a plurality of compounds for inhibition of binding between a ligand and an ABCA1 translation product, or a fragment, or derivative, or variant thereof, said assay comprising the steps of:

- (i) adding a liquid suspension of said ABCA1 translation product, or a fragment, or derivative, or variant thereof, to a plurality of containers;
- (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers;
- (iii) adding a detectable ligand, preferably a fluorescently labeled ligand to said containers;
- (iv) incubating said ABCA1 translation product, or said fragment, or derivative, or variant thereof, and said compound or compounds, and said detectable, preferably said fluorescently labeled ligand;
- (v) measuring amounts of detectable ligand or fluorescence associated with said ABCA1 translation product, or with said fragment, or derivative, or variant thereof; and

(vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said ABCA1 translation product, or said fragment, or derivative, or variant thereof.

17. An assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to an ABCA1 translation product, or to a fragment, or derivative, or variant thereof, said assay comprising the steps of:

- (i) adding a liquid suspension of said ABCA1 translation product, or a fragment, or derivative, or variant thereof, to a plurality of containers;
- (ii) adding a detectable compound, preferably a plurality of detectable compounds, in particular a fluorescently labeled compound or a plurality of fluorescently labeled compounds to be screened for said binding to said plurality of containers;
- (iii) incubating said ABCA1 translation product, or said fragment, or derivative, or variant thereof, and said detectable, preferably said fluorescently labeled compound or fluorescently labeled compounds;
- (iv) measuring amounts of detectable compound or fluorescence associated with said ABCA1 translation product, or with said fragment, or derivative, or variant thereof; and
- (v) determining the degree of binding by one or more of said compounds to said ABCA1 translation product, or said fragment, or derivative, or variant thereof.

18. A protein molecule shown in SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

19. A protein molecule shown in SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

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20. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a protein molecule shown in SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell.